Protocol Number: P2959



PROTOCOL



Protocol Number: P2959

GLP Study ID: GLP 2505

GLP Continuous Bacterial Reduction on Coated Surfaces

Test Microorganism(s)
Pseudomonas aeruginosa ATCC 15442

Data Requirement
U.S. EPA OCSPP 810.2300

Study Spansor BEHR 3400 W. Segerstrom Ave Santa Ana, CA, 92704

Testing Facility
Microchem Laboratory
1304 W. Industrial Blvd.
Round Rock, Texas 78681

<u>Author</u> Hillary Johnson, M.S.

<u>Date</u> 23SEP2020 Revised 01OCT2020

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I. Introduction

This document details the procedure for evaluating copper embedded antimicrobial surface coating products with continuous bacterial reduction claims as proposed by Corning Incorporated and the Study Sponsor and as recommended by the EPA. This document also explains the terms and conditions of testing.

II. Purpose

The purpose of this study is to document the efficacy of the test article against the test system (microorganisms) under the test parameters specified in this protocol.

III. Justification for the Selection of Test System (Microorganism)

The United States Environmental Protection Agency (US EPA) requires specific antimicrobial claims made for disinfectants sold in the United States to be supported by relevant test systems (microarganisms) as outlined in the United States Environmental Protection Agency Product Performance Test Guidelines, OCSPP 810.2300, Sanifizers for Use on Hard Surfaces – Efficacy Data Recommendations and other related EPA guidance.

IV. Terms and Conditions

Studies by Microchem Laboratory are conducted in accordance with general terms and conditions posted on www.MicrochemLab.com/terms

Prior to study initiation, Microchem Laboratory should receive the approved and signed protocol, test article and payment. Changes to the signed, approved protocol will require amendment and may incur additional fees. Cancellation of the study any time after study initiation may result in a cancellation fee of up to 100% of the total study cost, to be determined by laboratory management at its sole discretion.

Microchem Laboratory may repeat studies at its cost in the event of an unintended protocol non-conformance that affects the study outcome, or for studies which yield invalid control results. If the Sponsor requests a specific neutralizer to be utilized in testing and test controls indicate incomplete or inadequate neutralization, repeat testing will be at the Study Sponsor's expense for applicable testing. Repeat testing may be conducted under the current initiated protocol and Microchem Laboratory GLP study identification number. In addition, the Study Sponsor is responsible for the cost of all studies performed to confirm the outcome of a previous study and for ensuring that the study will meet their regulatory objectives.

The Study Sponsor must obtain written consent from Microchem Laboratory to use or publish its protocols, study reports (or parts thereof), logo or employee names for marketing purposes.

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V. Test Article Characterization and Handling

As stated in 40 CFR Part 160 Subpart F [160.105], each batch (lat) of test article shall be characterized as to identity, strength, purity, composition, and solubility (as applicable), and shall be documented prior to use in this assay. Stability of the test formula shall be determined prior to or concomitantly with this study. If the requirements set forth in 40 CFR Part 160 Subpart F [160.105] have not been met, this will be noted in the Good Laboratory Practice compliance statement in the study report. Certificates of Analysis (C of A) will be appended to the study report, if provided by the Study Sponsor.

Test articles are handled as follows:

- The test article is stored at ambient (room) temperature under fluorescent lighting or in a cabinet.
- The test article is shaken or otherwise mixed well immediately prior to use (if applicable).
- The test article is handled safely in accordance with the chemical risks it may pose, stated in the SDS or by the Study Sponsor during the course of pre-study communication.

VI. Study Dates

The listed proposed experimental start and completion dates are estimates based on the current laboratory schedule and may change based on when the test article, sponsor signed protocol, and payment (if applicable) are received at the testing laboratory. To avoid scheduling delays, assure that all paperwork is completed fully and accurately.

Proposed Experimental Start Date: Proposed Experimental Termination Date: 19OCT2020 02NOV2020

VII. Procedure for the Identification of the Test System

Microchem Laboratory maintains Standard Operating Procedures which outline the procedures for receipt, storage, and tracking of microorganisms. The vessels, racks, and trays containing the test system are labeled with microorganism identifiers to maintain microorganism traceability. Information regarding the microorganism identity, strain, propagation procedure, media utilized, etc. is documented in the study raw data. Following testing, the microorganism identity of positive test replicates is confirmed following the appropriate macroscopic, microscopic, and biochemical assays. All studies are assigned a unique identification number which is labeled on the test and control vessels, racks, trays, etc. Additionally, Standard Operating Procedures are also in place for the receipt, storage, and usage tracking of all test and control articles utilized in testing. These procedures are followed to identify and document the test system.

VIII. Test System (Microorganism)

Pseudomonas aeruginosa ATCC 15442 received from the American Type Culture Collection (ATCC).

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IX. Procedure

Throughout the procedure, the term "exposed" refers to carriers subjected to the simulated wear procedure and the term "unexposed" refers to carriers that were not subjected to the wear procedure. The term "treated carriers" refers to coated carriers that contain the active ingredient and the term "untreated carriers" refers to coated carriers that do not contain the active ingredient.

Carrier Requirements for Testing

Product Lot	Paint Type	Test Carriers per Paint Type	Control Carriers per Paint Type
Lot I	Eggshell Base Paint, Paint with Organic Pigment, Paint with Inorganic Pigment, Carbon Black Paint, and	3 unexposed, treated carriers 5 exposed to cleaner, treated test carriers 5 exposed to quat, treated test carriers	3 unexposed, untreated control carriers 3 exposed to cleaner, untreated control carriers 3 exposed to quat, untreated control carriers
Lot II	Semi-Gloss Deep Paint	5 unexposed, treated test carriers	3 unexposed, untreated control carriers
Viability (Reference) Control	Uncoated scrub chart		3 carriers

The Study Sponsor will provide Microchem with test articles that have been treated with the test substance. Records concerning the treatment of the carriers with the test substance will be maintained by the Study Sponsor and will not be included in the final report. The Study Sponsor will provide Microchem with the date test articles were treated and that date will be included in the final report. Test articles will be used within one week of treatment.

The Study Sponsor will perform the simulated wear of carriers following treatment of the test substance and the appropriate dry time. Records concerning the simulated wear procedure will be maintained by the Study Sponsor and will not be included in the final report. Product performance testing will be initiated within 5 days of the simulated wear process. Wear tests with one disinfectant/cleaning solution will be completed within one week from start to finish.

Preparation of Carriers upon Arrival at Microchem

- The cut carriers are visually screened for surface defects and edge abnormalities. Any carriers with visible surface or cut edge artifacts are discarded.
- Each carrier is physically screened to insure uniformity. Carriers with visible surface or edge abnormalities (e.g. chipping, gouges, pits or deep striations, etc.) are discarded. This screening will be conducted prior to the wear cycles.
- To decontominate the surface prior to testing, carriers are placed in a biological safety cabinet and exposed to UV light for 15 ± 2 minutes per side. Using forceps, each carrier is then placed in a sterile Petri dish, with the treated surface up.
- To ensure sterility, an untreated control and a treated test carrier for each batch are incubated in appropriate
 growth media at appropriate conditions. The sterility check should result in no microbial growth and will be
 included in the final report.
- Decontaminated carriers will be used within one week of preparation. Carriers will be stored in paper packaging
 covered by interleaf paper until use. All carriers are for single use only. Production lot (batch) identity will be
 maintained throughout the testing process.

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Preparation of Test Culture

- The test culture is initiated from a frozen single stock culture cryovial thawed to room temperature.
 - Frozen cryovials are made from lyophilized cultures from ATCC. Using a tube containing 5 6 ml of tryptic soy broth (TSB), aseptically withdraw 0.5 1.0 ml and rehydrate the lyophilized culture. Aseptically transfer the entire rehydrated pellet back into the original tube of broth and mix thoroughly. Incubate the broth culture at 36 ± 1°C for 24 ± 2 hours.
 - After incubation, streak a loopful of the suspension on tryptic say agar (TSA) to obtain isolated colonies.
 Incubate the plates at 36 ± 1°C for 18 24 hours.
 - Select 3-5 isolated colonies of the test organism and re-suspend in 1 ml TSB.
 - Select only golden yellow colonies.
 - Spread plate 0.1 ml of the suspension on each of 6 10 TSA plates. Incubate at 36 ± 1°C for 18 24 hours.
 - Following incubation of the agar plates, place approximately 5 ml sterile cryprotectant solution on the surface of each plate (TSB with 15% v/v glycerol). Re-suspend the growth in the solution using a sterile spreader without damaging the agar surface. Aspirate the suspension from the plate with a pipette and place it in a sterile vessel. Repeat the harvesting procedure with the remaining plates and continue adding suspension to the vessel. If more than one vessel is used, pool the vessels prior to aliquoting culture. Mix the contents thoroughly. Immediately after mixing, aliquot 0.5 1.0 ml of the harvested suspension into cryovials; these represent the frozen stock cultures.
- Defrost and briefly vortex cryovial to mix
- Test culture is initiated by transferring 0.010 ml from the thawed stock to a tube containing 10 ml of TSB and then
 vortex mixing. Test culture is incubated for 18 24 hours at 36 ± 1° C.
- Each test culture tube is gently vortex mixed and allowed to stand at room temperature for ≥ 10 minutes. Then the
 upper portion of the cultures are collected and pooled into an appropriate vessel(s).
- For the purpose of achieving carrier counts within the range of the study, dilute in Phosphate Buffered Saline (PBS) or concentrate the culture appropriately to achieve the target carrier counts (6 8 logs/carrier).
 - or concentrate the culture appropriately to achieve the target carrier counts (6 8 logs/carrier).

 Centrifuge at around 5000 gN for 20 ± 5 min and re-suspend the pellet in 10 ml PBS. Remove the supernatant without disrupting the pellet.
- Titer of final test culture (with soil load) is determined for informational purposes. Plate dilutions on TSA plates (or TSA with 5% sheep's blood) or other appropriate medium and incubate at 36 ± 1° C for 24 48 hours. Count the number of colonies to determine the number of organisms per ml (i.e. CFU/ml) of the inoculum present at the start of the test.

Supplementation of Test Culture with Organic "Soil" Load

- The soil load consists of a 5% fetal bovine serum (FBS) and 0.01% Triton X-100 solution, unless atherwise requested by the Study Spansor.
- Following the addition of soil load, the final test suspension is vortex mixed for 10 seconds immediately prior to

Efficacy Test Procedure

- · Aseptically transfer each carrier into a sterile Petri dish.
- Coated control carriers should be evaluated concurrently with the coated test carriers.
- The contact time begins immediately upon inoculation of the carrier surface. The temperature and relative humidity are recorded after inoculation of the first carrier.

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- A 0.020 ml volume of test culture is spread across the entire surface of the carrier, using a sterile bent pipette tip,
 without allowing the test culture to touch the edges of the carrier. This is repeated for each carrier at an interval
 that ensures careful and aseptic handling of the carriers. Record the temperature and relative humidity following
 inoculation of the final carrier.
- Carriers are to remain in a horizontal position under ambient conditions, with the lid on the Petri dish for the
 duration of the contact time (120 ± 5 minutes).
- Following completion of the contact time, sequentially and aseptically transfer each carrier into 20 ml of Letheen broth (neutralizer solution). This represent the 10° dilution. Record the temperature and humidity when neutralizing the first and last carrier.
- After all carriers have been transferred into the neutralizer, sonicate in a water bath for 5 minutes ± 30 seconds.
 Each liquid level in the tubes should be even with the liquid level in the bath. The tubes are not allowed to touch the bottom or sides of the ultrasonic water bath.
 - The frequency of the sonicator will be recorded in the raw data and included in the final report.
- Within 30 minutes of sonication, prepare serial dilutions of the neutralized solution (10°) out to the 10° for the treated carriers.
- Transfer the coated control carriers to neutralizing subculture medium and plate the appropriate dilutions in duplicate to yield counts of 10 – 300 per plate.
- Plate 1.0 ml aliquots of the 10° dilution and 0.10 ml aliquots of the 10° 10° dilutions on TSA plates in duplicate using standard spread plating technique.

Neutralization Confirmation

- Perform a neutralization confirmation control to demonstrate the neutralizer's ability to inactivate the test carrier
 prior to or concurrently with the test. The neutralization of the coated test carriers is confirmed by using treated test
 and untreated control carriers and the neutralizer as in the test procedure.
- . The neutralization assay control is performed in triplicate/lot for the test surface and the control.
- The ambient temperature and relative humidity is recorded and included in the final report.
- A test carrier is placed in a tube containing 20 ml of neutralizer solution. This is performed with 3 test carriers per lot.
- . The carrier is allowed to rest in the neutralizer solution for approximately 10 minutes.
- Add a 1.0 ml aliquot of a diluted suspension of test microorganism yielding 10 100 CFU/0.1 ml of neutralizing subculture medium to the neutralizer and mix well.
- The suspension is allowed to rest for approximately 10 minutes.
- A 0.1 ml of the mixed solution is plated in duplicate using spread plating techniques on plates with TSA (or TSA with 5% sheep blood).
- A numbers control (provides baseline level of CFU for comparative purposes) is performed using untreated control
 carriers; the process is the same as indicated for the test carriers.
- The resulting plates are incubated as in the test and enumerated.

Carrier Sterility Control(s)

 A representative un-inoculated coated test and coated control carrier are each placed in neutralizer solution, sonicated and plated as in the test, then incubated alongside the test materials to confirm sterility at the time of test.

Media Sterility Control(s)

- An aliquot of the organic soil load is added to sterile growth medium and incubated alongside the test materials to confirm sterility at the time of test.
- An aliquot of the neutralizer is added to sterile growth medium and incubated alongside the test materials to confirm sterility at the time of test.

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 A plate containing only growth medium used in this study is incubated alongside the test materials to confirm sterility at the time of test.

Viability and Culture Purity Controls

- A volume of each test microorganism culture is streaked to the appropriate growth agar to achieve isolated
 colonies in order to confirm culture purity and media viability.
- Inoculated scrub charts carriers are used to verify the test organisms ability to survive on inert surfaces under the
 test conditions. Following completion of the exposure time, control carriers will be transferred to neutralizing
 subculture media and sonicated as in the test. Ten-fold serial dilutions of the neutralizing subculture medium will
 be prepared and 1.0 ml or 0.1 ml aliquots of the appropriate dilutions will be spread plated in duplicate to yield
 countable numbers. The resulting plates are incubated as in the test and enumerated.
- Prepare and plate serial dilutions of the culture used as inoculum. Incubate the resulting plates as in the test and
 then count the colonies to determine the number of organisms per ml of inoculum present at the start of the test.

Incubation of Test Materials

- Incubate the plates at 36 ± 1°C for 48 ± 4 hours.
 - Following incubation, colonies are counted and results are recorded. If colonies can not be counted following the 48 ± 4 hours incubation period, plates may be stored at $2-8^{\circ}\text{C}$ for up to 5 days prior to reading results.

X. Calculations

- The mean log reduction in viable cells for each microbe is calculated for the following treatments: 1) exposed treated test carriers for production lot 1, 2) unexposed treated test carriers (one 3-carrier set per microbe) for production lot 1 and 3| unexposed treated test carriers for production lot 2.
- Log reduction values are calculated based on the difference in log densities associated with the exposed/unexposed treated test carriers compared to the untreated control carries.
- Dilutions will be counted and recorded in the raw data including counts of "0," and excluding dilutions with counts
 of ">300." Dilutions demonstrating colony counts >300 may be noted as too numerous to count (TNTC).
- Efficacy results are reported as the percent reduction of the geometric mean of the test microorganism on the
 exposed treated test carriers calculated relative to the geometric mean of the test microorganism on the
 unexposed, untreated control carriers. An assessment is also made between exposed untreated test carriers and
 unexposed untreated control carriers.

Viable bacteria per carrier is calculated as follow:

CFU/Carrier = (Average CFU/plate x dilution factor (relative to carrier) × 20 ml) / volume plated

Percent Reduction is calculated as follows:

Geometric Mean of Untreated Control Carriers = Antilog $(log_{10}X_1 + log_{10}X_2 + log_{10}X_3)$

X= the Number of Microorganisms Surviving Per Control Carrier

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Geometric Mean of Treated Test Carriers = Antilog $(log_{10}Y_1 + log_{10}Y_2 + log_{10}Y_3 + log_{10}Y_4 + log_{10}Y_5)$

Y= the Number of Microorganisms Surviving Per Test Carrier

Percent Reduction = ((A-B)/A) x 100

A = Geometric Mean of the number of microorganisms surviving on the untreated control carriers

B = Geometric Mean of the number of microorganisms surviving on the treated test carriers

Log₁₀ Difference = Log₁₀A - Log₁₀B

A = Geometric Mean of the number of microarganisms surviving on the untreated control carriers

B = Geometric Mean of the number of microorganisms surviving on the treated test carriers

Neutralization Confirmation Calculations are as follows:

Logio Difference = LogioA - LogioB

A = Geometric Mean of the number of microorganisms surviving on the numbers control

B = Geometric Mean of the number of microorganisms surviving on the neutralization control

X. Proposed Statistical Analysis

Not applicable.

XI. Methods for the Control of Bias

Not applicable.

XII. Success Criteria

The experimental success (controls) criteria follow:

- All media sterility controls must be negative for growth.
- · Carrier sterility control must be negative for growth.
- The media viability control must be positive for growth.
- · All test microorganisms must demonstrate culture purity.

Neutralization is validated if:

- The neutralization confirmation final suspension yields an average concentration of 10-100 CFU
- The neutralization test suspension demonstrates is ≤50% of the difference between the treated and control
 carriers, demonstrating that the neutralizer is effective.
- The mean log reduction values (per abrasion/chemical treatment per microbe) for the exposed treated test carriers compared to the unexposed treated test carriers should be within 0.5 log; in addition, the mean log reduction for the exposed product carriers should not be less than the performance standard of 3 logs for any abrasion/chemical treatment group for either of the test microbes.
- The scrub chart carrier viability control must demonstrate 6 8 logs CFU/carrier.
- · There is no acceptance criterion defined for the initial suspension control (inoculum count).

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XIII. Product Performance Criteria

The Environmental Protection Agency performance criteria follow:

A ≥99.9% reduction (≥3 log reduction) in the numbers of each test microorganism when compared to the control.

XIV. Reporting

Results are reported accurately and fully, in accordance with Environmental Protection Agency GLP (40 CFR Part 160). A draft report may be provided for review by the Study Sponsor prior to study completion.

XV. Data and Sample Retention

- The original (or certified copy) of the study report, protocol, and corresponding raw data will be held in the archives of Microchem Laboratory indefinitely. For studies not meeting the performance criteria for submission or for studies that have been canceled prior to the generation of valid data, the original (or certified copy) of the final study report, protocol, and corresponding raw data will be held in the archives of Microchem Laboratory for a minimum of two years following the study completion date at which time they may be removed from the archive or transferred to the Sponsors archive at their expense.
- If requested by the Study Sponsor (ar Sponsor Representative), the study file may be transferred to the Study Sponsor's archive at the Study Sponsor's expense prior to the time frames listed.
- All test facility records including, but not limited to, standard operating procedures, quality assurance inspection records, temperature and equipment records including maintenance, inspection and calibration, and employee training records will be maintained at Microchem Laboratory indefinitely.
- The test article (or test control, test article, test device, as applicable) may be returned to the Study Sponsor's request and expense following study completion unless otherwise requested to be returned earlier. If the Study Sponsor does not request return of the sample, it will be disposed >90 days following the study completion. Arrangements may be made for extended storage as necessary, at the Sponsor's request and expense.

XVI. Quality Assurance

The study is conducted in accordance with Microchem Laboratory's Quality Management System and EPA 40 CFR Part 160 and will undergo a full quality assurance review. All protocol amendments will be fully recorded and reported, as well as any deviations from the protocol.

XVII. References

- "ASTM International" ASTM Official Method E1153-14. 2014. Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate, Hard, Nonporous Non-Food Contact Surfaces.
- "ASTM International" ASTM Official Method E1054-08. 2013. Standard Test Method for Evaluation of Inactivators
 of Antimicrobial Agents.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance
 Test Guidelines OCSPP 810.2000: General Considerations for Testing Public Health Pesticides Guidance for
 Efficacy Testing. February 2018.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2300: Sanitizers for Use on Hard Surfaces. – Efficacy Data Recommendations. February 2018.
- Protocol for Continuous Bacterial Reduction on Coated Surfaces, provided by Corning Incorporated and reviewed and approved by the US EPA.

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Specific Testing Parameters to be completed by the Study Sponsor/Representative - all fields need to be completed before testing may commence

Test Article Name	2190	3193	
Test Article Batch Numbers	150036-2050-CUGLASS EX 51 150036-2050-GLOLASS EX 52	150036-3300-CU Ex.21 (N/a.i.)	
Manufacture Date(s)	10+#1 9/22/2020	10+ #1: 9/21/2020 10+#2: 9/22/2020	
Expiration Date(s)	Let #11 9/22/2022 Let #2; 9/23/2022	10t #1: 9/21/2022 1ct #2: 9/22/2022	
Test Article Shipment Status	□ Use test article already present at Microchem. Test article ## ill be shipped. Estimated arrival date, if known:		
Test Article Storage	© Room temperature (default for all packages unless other-tise advised) □ 2 8 C □ Other:		
Test Article Hazards	☑ None knot □ SDS ill be provided □ Other:		
Test Article Active Ingredient			
Active Ingredient Level	☑ At or below Lower Certified Limit	(LCL) At or below nominal	
Active Ingredient Concentration as submitted (for neutralization information only, not for chemical characterization)	0.96% by	weight	
Test Article Size	⊠1 x 1 □ Other:		
Organic Soil Load	☐ 5½ fetal bovine serum and 0.01% Triton №100 solution ☐ None ☐ Other:		
Exposure Temperature	® Room Temperature (15-30°C) □ Other:		
EPA 40 CFR Part 160.31(d) requires testing facility management to assure that the test, control, and reference substances have been appropriately tested for identity, strength, purity, stability and uniformity, as applicable.	Applicable identity, strength, purity, stability, and uniformity testing has been or will be completed prior to efficacy testing: Performed under 40 CFR Part 160 regulations. Yes M No Stability testing has been or will be completed prior to efficacy testing or concomitantly with efficacy testing: Performed under 40 CFR Part 160 regulations. Yes M No Performed under 40 CFR Part 160 regulations. Yes M No If no is marked, compliance status will be noted in the GLP compliance statement in the final report. Yes M No To make the marked as much as the final report. Yes M No If no is marked, compliance status will be noted in the GLP compliance statement in the final report. Yes M No Yes M No		



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Certificate of Analysis (CoA)	□ CoA for each batch provided. CoA ill be appended in the final report. □ CoA ill not be provided. Le cuit provide the further that QC date		
Protocol Modifications			
Regulatory Agency(s) that report may be submitted to	MEPA □ Health Canada		

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/III. Authorized Personnel		
 Due to Microchem Laboratory confidentiality policy, stuc Sponsor/Sponsor Representative who has signed the proto- additional personnel authorized to receive information regar 	col unless atherwise noted in w	
1. GREG SARNECKI		
2. BILL SCHWINGEL		
3. MYLINH PHAM		
4.		
X. Protocol Approval		
"I, the Study Spansor, have read and understand the study prof information and parameters accurately describe the test(s) to Practice Standards (GLPS) stipulated by 40 CFR 160. I have conditions listed in the protocal." Study Spansor/Representative Signature Approving Protocol	be completed in accordance w	ith Good Labora
John A. Gilbert Study Spansor/Spansor Representative Printed Name		
Ol o o		
Study Sponsor/Sponsor Representative Signature	Oct 2,	2020
Study Sponsor/Sponsor Representative Signature	Date	
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Microchem Laboratory Study Director		
Microchem Laboratory Study Director Hillary Johnson Study Director Printed Name		
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